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Exploratory surveys in Australia and Asia for natural enemies of Old World climbing fern, *Lygodium microphyllum*: Lygodiaceae

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Abstract

Lygodium microphyllum, Old World climbing fern, is native to the wet tropics and subtropics of the Old World and an invasive weed in southern Florida. Exploration for natural enemies of this weed was conducted between 1997 and 2002 in Australia, China, India, Indonesia, Japan, Malaysia, New Caledonia, Singapore, Taiwan, Thailand, and Vietnam. Two species of mites and 20 insect species were collected. The eriophyid mite, *Floracarus perrepae* Knihinicki and Boczek, was the most widely distributed and appeared to cause significant damage to the plant over time. Several unique geographical genotypes of *F. perrepae* were identified. Other promising candidates for further research are the musotimine pyralid species, *Neomusotima conspurcatalis* Warren, *Cataclysta camptozonale* Hampson, *Cataclysta* sp. 2, and *Musotima* sp., all leaf-feeders, as well as the stem-borer *Ambia* sp. All of the herbivores collected, except the eriophyid, typically occurred at low field densities. The pyralid species may reach high densities and cause great damage in Florida in the absence of their co-evolved natural enemies. The mite *F. perrepae* shows the greatest potential for biological control based on field surveys and initial laboratory observations; however, future studies must identify mite genotypes that are best adapted to the invasive Florida form of the fern.
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Keywords: *Floracarus*; Fern-feeding moths; Invasive weed; Florida everglades; Foreign exploration; Biological control; Agent selection

1. Introduction

Lygodium microphyllum (Cav.) R. Br. (Lygodiaceae, Pteridophyta), the Old World climbing fern, is native to the Old World wet tropics and subtropics of Africa, Australasia, Asia, and Oceania (Pemberton, 1998). It is an aggressive invasive weed in moist habitats of southern Florida (Pemberton and Ferriter, 1998) and is classified as a Category I invasive species by the Florida Exotic Plant Pest Council (Langland and Craddock Burks, 1998). It was first found to be naturalized in Florida 1965, however, its explosive growth and rapid spread is now of increasing concern because of its dominance over native vegetation. Over 43,000 ha (107,000 acres) are now infested in south Florida, a 150% increase from 1997 to 1999 (Pemberton et al., 2002). Pemberton and Ferriter (1998) predict the plant could spread further north in Florida into areas with low winter temperatures (USDA Plant Hardiness Zone 9b) (Cathey, 1990). Warm areas with high rainfall bordering the Gulf of Mexico and the Caribbean such as southern Louisiana, Yucatan of Mexico, Cuba, and Puerto Rico, would also be suitable for *L. microphyllum*. Herbicidal and mechanical controls are expensive, have limited effectiveness, and can cause considerable damage to non-target plants. Because *L. microphyllum* has become so invasive in south Florida, with a strong potential to spread further, a biological control program was initiated in 1997.

This paper covers exploration activities in Australia and Asia for biological control agents of *L. microphyllum*. Most of the information in this paper comes from

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surveys conducted in Australia and Southeast Asia from 1999 to 2002. *L. microphyllum* was found in a variety of subtropical and tropical habitats throughout its range–from 29°S in New South Wales, Australia, through Southeast Asia, to 24°N in the Guandong Province of China. This entire range was prioritized for exploration because the plant was common and not considered to be weedy; parts of the region are climatically similar to Florida; and a preliminary survey in Japan and Hong Kong in 1997 indicated the presence of herbivorous insects attacking *Lygodium* spp. The decision to explore this area was later bolstered by preliminary molecular evidence, which indicated that the invasive population in Florida was similar to collections from Australia and Southeast Asia and most distant from African populations (Goolsby and Pemberton, unpublished data). Populations of *L. microphyllum* were compared using gene sequences from the chloroplast intron using the methods of Thomson (2000). Although *Lygodium* is a worldwide genus, 15 of the 26 species occur in the Australian and Southeast Asian region (Garrison Hanks, 1998), including *Lygodium flexuosum* (L.) Sw. (Lygodiaceae), *Lygodium reticulatum* Schkuhr (Lygodiaceae), *Lygodium japonicum* (Thunb.) Sw. (Lygodiaceae), and *Lygodium circinnatum* (Burm.) S (Lygodiaceae). The high diversity of *Lygodium* species in this region should support more specialist herbivores than other areas. Pemberton did the first survey in Hong Kong and the later work in Japan and Taiwan. Goolsby focused his surveys on Australia, New Caledonia, and India and led the preliminary host screening. The Southeast Asian surveys were conducted by A.D. Wright.

2. Materials and methods

2.1. Exploration

Selection of search areas was guided by climate matching, habitat diversity, herbarium locality data, and knowledgeable specialists. The following countries were visited between September 1997 and March 2002: Australia, China, Japan, Indonesia, Malaysia, New Caledonia, Singapore, Taiwan, Thailand, and Vietnam. One preliminary survey was conducted in India in May 2002; it is reported at the end of this paper. CLIMEX software (Sutherst et al., 1999) was used to match the climate of West Palm Beach in South Florida with locations within the native range of *L. microphyllum* in Australia and Southeast Asia. Each survey at each site was documented with a unique Australian Biological Control Laboratory (ABCL) collection number and the following data were recorded: date, time spent searching, site coordinates, temperature, plant growth characteristics, and herbivore species. (This information can be obtained from ABCL, or through the USDA-ARS documentation Center that holds copies of ABCL annual reports.) Most locations were visited several times to account for seasonal effects on herbivore biodiversity. In addition to *L. microphyllum*, we surveyed *L. reticulatum* in Australia (21) and New Caledonia (9), and *L. flexuosum* (65) and *L. japonicum* (18) in Asia. Intensive monthly surveys were conducted from February 2000 to March 2002 at four field sites with *L. microphyllum* in southeast Queensland. Methods for collection included hand searches, sweeping, beating of foliage, and dissection of underground plant parts.

2.2. Identification and characterization of arthropods

Arthropods collected in the surveys were initially characterized with molecular tools and by comparison with specimens in the ABCL collection. Selected specimens were forwarded to cooperating systematists at the ARS Systematic Entomology Laboratory (SEL) (Beltsville, MD, USA), Queensland Museum (Brisbane, Queensland, Australia), Australian National Insect Collection (ANIC) (Canberra, ACT), and/or the Natural History of London (London, UK) (NHM) and the National Museum Prague, Czech Republic. Representatives of each insect and mite species collected were characterized at the CSIRO Entomology Molecular Diagnostic Laboratory, (Canberra, ACT). Multiple specimens were characterized in order to ensure consistency in the results. The D2 expansion domain of the 28S rRNA isolated from the nuclear genome was sequenced. The methods are those described by DeBarro et al. (2000). The numbers of individuals sequenced ranged from 1 to 30 depending on the quantity available. Whenever possible, we attempted to characterize individuals from each unique collection location and host plant. We used molecular characterization as a tool while taxonomic identifications were proceeding and as an interim method for assessing species diversity, identifying cryptic species, and matching immature stages with adults.

2.3. Preliminary host-range testing

A protocol was developed for preliminary host-range testing of the Pyralidae collected in the survey. Twelve plant species were tested in no-choice tests. Two pairs of newly emerged moths were placed inside a moistened plastic bag that enclosed a single, live fern leaf, or small group of pinnae. Adults were left in the bags until death or for five days, if eggs were visible. Larvae from these eggs were allowed to complete development on the live host plant. Test plants with adult moths and/or developing immatures were held at 25 ± 2°C with natural day lengths.

3. Results

3.1. Foreign exploration

Lygodium microphyllum was found growing in a variety of habitats across its native range. In the states of Queensland and New South Wales in eastern Australia, *L. microphyllum* is common in the freshwater creeks and depressions of the coastal wetlands, often growing with *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae). In tropical north Queensland, *L. microphyllum* is sympatric with *Lygodium reticulatum*, but the latter prefers the upland rainforest habitats. In the monsoonal climate of far north Australia (Western Australia and Northern Territory), *L. microphyllum* is sympatric with *Lygodium flexuosum* and grows in the forest patches and along perennial creeks with abundant, slow-moving fresh water. In the Kimberley range of Western Australia, *L. microphyllum* is found growing in sheltered canyons near permanent springs. Across its range in Australia, *L. microphyllum* regrows vigorously following periodic bushfires, utilizing nutrients during a period of low herbivory. In Southeast Asia, *L. microphyllum* is common along the edges of lowland rainforests in peat soils, in coastal wetlands, and in wet, lateritic clay soils. Near the northern limit of its range in southern China, *L. microphyllum* is sympatric with *L. japonicum* and *L. flexuosum*. *L. japonicum* appears to be more competitive in the drier rocky soils, often found growing in a low prostrate form over whole hillsides. Throughout its range in Australia and Asia, the plant was never found to be more than a few meters tall and did not dominate its plant community.

Two mites and 20 insect species were collected feeding on *L. microphyllum* or related species (Table 1). The most commonly collected and geographically widespread herbivore was the eriophyid mite *Floracarus perrepae* Knihinicki and Boczek, followed by the pyralid moth *Neomusotima conspurcatalis* Warren. All of the herbivores on the list, except the eriophyid, typically occurred at low field densities.The total number of collections in which each species was found or showed evidence of its distinct damage was summarized for 513 field collections. *F. perrepae* were collected from 172 (or 33.5%) of the 513 samples (Fig. 1), as contrasted with an almost equal number of collections, 154 (or 30.0%) from which no herbivores were recorded at all. In nearly half of the collections, 248, either no insect herbivores or only *F. perrepae* were collected. Several Lepidoptera species were collected and were represented in 41.4% of the samples (Fig. 1). The 22 herbivore species listed in Table 1 were collected over a three-year period, primarily in Australia and Southeast Asia.

The most promising species collected in the surveys were taken to ABCL for preliminary host-range testing. Closely related ferns, including related *Lygodium* species from the Neotropics, were tested (Table 2). The known biology, host range, and potential of these arthropods as biological control agents are discussed in the next section in the approximate order of value as biological control agents.

Comparison of climates using CLIMEX is summarized in Fig. 2. Based on the simple comparison of average monthly maximum and minimum temperatures, rainfall amounts, and rainfall pattern and relative humidity, eastern Australia, southern China, and southeast Africa had the best climatic matches with southern Florida, where selected agents from this survey are likely to be released.

3.2. Herbivores collected in surveys

3.2.1. *Floracarus perrepae* (Acariformes: Eriophyidae)

Floracarus perrepae Knihinicki and Boczek is the most widespread arthropod associated with *L. microphyllum*. It has been collected from locations in Australia, China, India, Indonesia, Malaysia, New Caledonia, Singapore, Sri Lanka, and Thailand. Knihinicki and Boczek (2002) included specimens from Australia, China, and New Caledonia in the original description of the species. Populations of this mite from five locations in Australia and Thailand, compared by sequencing the nuclear 28S rRNA of D2 gene, had the same gene sequence. *F. perrepae* from China and New Caledonia showed 10 and two base-pair changes for the same gene region, respectively, which indicates there are unique genotypes of the mite within its distribution.

Floracarus perrepae appears to be tolerant of the wide range of climates found in the tropics and subtropics of the region. Populations of the mite are active year round. Mature females prefer the new sterile pinnae (the leaflets that do not produce spores) on actively growing plant tips for oviposition. Observations of newly formed leaf curls revealed up to five adults inside. Deformation in the leaf tissue appears to be induced by the feeding of the adult mite. Plant tissues become swollen and succulent as curling of the leaf begins. Attack on the leaf margin induces rolling, and on some pinnae, the whole margin may be affected. The leaf curls over downward and inward rolling on itself two–three times. Eggs are deposited and nymphs complete their development within the curl. Feeding by the adults and immatures leads to leaf necrosis and premature defoliation of *L. microphyllum* pinnae, gradually debilitating the plant over time. Eventually the mite-induced leaf necrosis and death equals the level of new growth. This ‘steady state’ is interrupted by the periodic bushfires in Australia. After a fire, the plant grows rapidly, stimulated by nutrients and apparent lack of suppression by the mite. Numerous field observations of *L. microphyllum*, after fire and without the mite, support the hypothesis that *F. perrepae* has a substantial impact on *L. microphyllum* (Goolsby, unpublished data).

Table 1
Herbivores collected from *Lygodium* spp. in Asia and Australia

Name (collection #)	Collection locations	Host plant
Floracarus perrepae Knihinicki and Boczek Acarina: Eriophyidae (2001224)	Australia, China, India, Indonesia, Malaysia, New Caledonia, Singapore, Sri Lanka, Thailand	*L. microphyllum* (Cav.) R. Br. *L. reticulatum* Schkuhr
Brevipalpis sp. Acarina: Tenuipalpidae (2001566)	China, Singapore, New Caledonia	*L. microphyllum* (Cav.) R. Br. *L. japonicum* (Thunb.) Sw.
Neomusotima conspurcatalis Warren Lepidoptera: Pyralidae (1999228)	Australia (Queensland, Northern Territory, Western Australia), China, Indonesia, Malaysia, Singapore, Thailand	*L. microphyllum* (Cav.) R. Br.
Cataclysta camptozonale Hampson Lepidoptera: Pyralidae (2000223)	Australia (Queensland)	*L. microphyllum* (Cav.) R. Br.
Cataclysta sp. B Lepidoptera: Pyralidae (1999231)	Australia (Queensland)	*L. microphyllum* (Cav.) R. Br. *L. reticulatum* Schkuhr
Musotima sp. Lepidoptera: Pyralidae (199704)	Malaysia, Singapore, Thailand	*L. microphyllum* (Cav.) R. Br.
Neomusotima fuscolinealis Yoshiyasu Lepidoptera: Pyralidae (1997650)	Japan	*L. japonicum* (Thunb.) Sw.
Pyraustinae sp. Lepidoptera: Pyralidae (2002566)	New Caledonia	*L. microphyllum* (Cav.) R. Br.
Ambia sp. Lepidoptera: Pyralidae (2001449)	Singapore, Thailand	*L. microphyllum* (Cav.) R. Br. *L. flexuosum* (L.) Sw.
Callopistria sp. A Lepidoptera: Noctuidae (2000686)	Australia (Queensland), China, India, Thailand	*L. microphyllum* (Cav.) R. Br.
Callopistria sp. B Lepidoptera: Noctuidae (2000280)	Australia (Northern Territory)	*L. microphyllum* (Cav.) R. Br.
Callopistria sp. C. Lepidoptera: Noctuidae (1998421)	Thailand	*L. microphyllum* (Cav.) R. Br.
Spodoptera litura (F.) Lepidoptera: Noctuidae (1996201)	Australia	*L. microphyllum* (Cav.) R. Br.
Archips machlopis Meyrick Lepidoptera: Tortricidae (1998421)	Malaysia, Singapore, Thailand	*L. microphyllum* (Cav.) R. Br.
Neostromboceros albicomus (Konow) Hymenoptera: Tenthridinidae (2001429)	Malaysia, Singapore, Thailand, Vietnam	*L. microphyllum* (Cav.) R. Br. *L. flexuosum* (L.) Sw.
Metriona sp. Coleoptera: Chrysomelidae (1999246)	Australia (Northern Territory, Western Australia)	*L. microphyllum* (Cav.) R. Br.
Endelus bakerianus Obenberger Coleoptera: Buprestidae (1999440)	Singapore, Thailand	*L. microphyllum* (Cav.) R. Br.
Manobia sp. Coleoptera: Chrysomelidae (1999432)	Thailand	*L. flexuosum* (L.) Sw.
Lophothetes sp. Coleoptera: Apionidae (1998550)	Palau	*L. microphyllum* (Cav.) R. Br.
Acanthuchus trispinifer (Fairmaire) Hemiptera: Membracidae (1999202)	Australia (Queensland, Northern Territory)	*L. microphyllum* (Cav.) R. Br.
Pseudococcus longispinus (Targioni-Tozzetti) Homoptera: Pseudococcidae (2000285)	Australia (Queensland)	*L. microphyllum* (Cav.) R. Br.
Octothrips lygodii Mound Thysanoptera: Thripidae (2000677)	China, Singapore, Thailand	*L. microphyllum* (Cav.) R. Br. *L. flexuosum* (L.) Sw. *L. japonicum* (Thunb.) Sw.

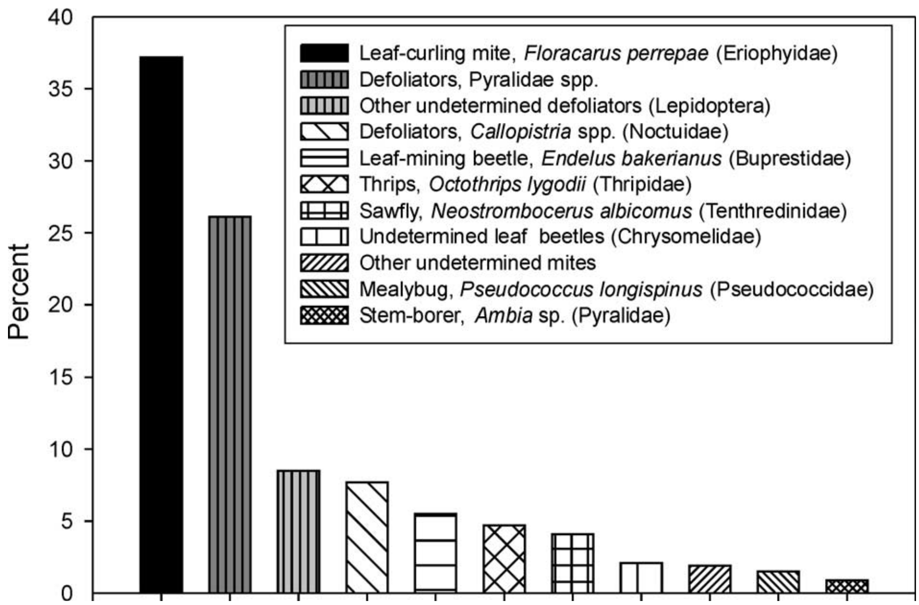

Fig. 1. Relative abundance of herbivores in field collections, based on 100% of the number of herbivores.

Table 2
List of species tested in Australia

Family	Plant species	Comments
Lygodiaceae	*Lygodium microphyllum* (Cav.) R. Br.	Australian form
Lygodiaceae	*Lygodium microphyllum* (Cav.) R. Br.	Florida form
Lygodiaceae	*Lygodium flexuosum* (L.) Sw.	Native to Australia and Asia
Lygodiaceae	*Lygodium japonicum* (Thunb.) Sw.	Native to Australia and Asia, weed in USA
Lygodiaceae	*Lygodium reticulatum* Schkuhr	Native to Australia and New Caledonia
Lygodiaceae	*Lygodium oligostachyum* (Willdenow) Desv.	Native to West Indies
Lygodiaceae	*Lygodium palmatum* (Bernhardi) Swartz	Native to temperate N. America
Lygodiaceae	*Lygodium venustrum* Sw.	Native to West Indies, Central and South America
Schizaeaceae	*Schizaea bifida* Willdenow	In same habitat in Australia
Schizaeaceae	*Anemia adiantifolia* (L.) Swartz	In same habitat in Florida
Aspleniaceae	*Asplenium nidus* L.	Ornamental
Aspleniaceae	*Asplenium scolopendrium* L.	Ornamental
Blechnaceae	*Blechnum indicum* Burm. f.	In same habitat in Australia
Cyathaceae	*Cyathea cooperi* (F.J. Muell.) Domin	Ornamental, native to Australia
Dennstaediaceae	*Hypolepis muelleri* N.A. Wakoef	In same habitat in Australia
Dennstaediaceae	*Pteridium aquilinum* (L.) Kuhn	Wide-spread, native to south Florida
Dryopteridaceae	*Nephrolepis biserrata* (Swartz) Schott	Native to Florida, ornamental
Dryopteridaceae	*Polystichum acrostichoides* (Michaux) Schott	Native to Florida
Dryopteridaceae	*Rumohra adiantiformis* (G. Forst.) Ching	Important florist fern, ornamental
Gleicheniaceae	*Sticherus flabellatus* (R. Br.) H. St. John	In same habitat in Australia
Lycopodiaceae	*Lycopodiella cernua* (L.) Pic. Serm.	Native to Florida and Australia
Ophioglossum	*Ophioglossum petiolatum* L.f.	Native to Florida and Australia
Osmundaceae	*Osmunda regalis* L.	Native to Florida, ornamental
Parkeriaceace	*Ceratopteris thalictroides* (L.) Brongniart	In same habitat in Australia
Polypodiaceae	*Phlebodium aureum* (L.) J. Smith	Native to Florida, ornamental
Polypodiaceae	*Platycerium hillii* T. Moore	Ornamental, native to Australia
Psilotaceae	*Psilotum nudum* (L.) Palisot de Beauvois	Native to Florida and Australia
Pteridaceae	*Acrostichum speciosum* Willdenow	Native to Australia
Pteridaceae	*Adiantum capillus-veneris* L.	Native to Australia and USA
Salviniaceae	*Salvinia molesta* D.S. Mitchell	Weed in Australia and USA
Selaginellaceae	*Selaginella emmeliana* F. Schmitz and M. Moller	Primitive fern ally
Thelypteridaceae	*Thelypteris patens* (Swartz) Small	Native to south Florida

The field host range of this species appears to be limited to *L. microphyllum* and *L. reticulatum*. Field collections of *L. japonicum*, *L. flexuosum*, and *L. circinnatum* do not show any evidence of this eriophyid mite. *F. perrepae* appears to be an excellent candidate for biological control based on its ability to

Fig. 2. A CLIMEX map showing the similarity of West Palm Beach, Florida with locations in the native range of *L. microphyllum*. Dots represent a 70% climate match or better.

suppress plant growth and its narrow host range. However, recent observations of Queensland population of the mite indicated that the mites performed poorly on the Florida form of *L. microphyllum* compared to the Queensland form of *L. microphyllum* (Goolsby, unpublished data). This suggests the genotypes may be host races of the mite. Searches for a *F. perrepae* race or biotype that is better suited to the Florida genotype of *L. microphyllum* are underway.

3.2.2. *Brevipalpis sp. (Acariformes: Tenupalpidae)*

Brevipalpis sp. was collected from *L. microphyllum* and *L. japonicum* in Australia, China (Hong Kong), New Caledonia, and Singapore. Feeding by the mites on the surface of the leaves caused a stippling effect. The damage to the plant appeared to be very minor. In New Caledonia we observed a similar stipling effect on a species of sword fern, *Nephrolepis* sp., that was growing with *L. microphyllum*. Due to its potentially broad host range and minor effect on the plant, this species was not considered further as a biological control agent.

3.2.3. *Neomusotima conspurcatalis (Lepidoptera: Pyralidae)*

Neomusotima conspurcatalis Warren has been collected throughout the range of *L. microphyllum* in Southeast Asia and Australia, except subtropical Queensland and New South Wales. Representatives from throughout its geographic range were compared using molecular diagnostic methods, which showed an exact match between the specimens from Australia, China, and other parts of Southeast Asia. It is not known why this species is not present in subtropical Queensland and New South Wales. It is common in other subtropical parts of Asia, including southern China, where winter minimum temperatures are just as cold as in subtropical Australia. The dry tropics between Townsville and Mackay, Queensland, from which *L. microphyllum* is absent, may act as biogeographic barrier that limits the southern distribution of the moth.

Adults of *N. conspurcatalis* are dark brown with distinctive lighter colored 'boomerang' shaped markings on their forewings, which are approximately 6 mm long. Eggs are yellow and laid singly or in small clusters, mostly on the new growth. Larvae are green with setae not visible to the naked eye. Early instar larvae skeletonize *L. microphyllum* leaves, while fourth-instar larvae consume entire leaves. *N. conspurcatalis* pupates above ground on the plant in a concealed location. The developmental period from egg to adult at 25 °C is approximately 25 days. Natural populations remain at very low levels, sometimes at undetectable levels, throughout the year. This was the most frequently collected insect species in the survey. Some collections were made from remote stands and isolated patches of new growth following extensive bush fires, which suggests good dispersal ability.

In preliminary host range testing, complete development occurred only on four of the six *Lygodium* spp. (Table 3). Females were induced to lay eggs on eight fern species outside the *Lygodium* genus, but none of the larvae lived past the first instar. The preliminary testing suggested that *N. conspurcatalis* may be a genus-level specialist. Neither the moth nor its distinctive feeding damage was observed in the field on *L. japonicum*, *L. reticulatum*, *L. flexuosum*, or *L. circinatum*. The lack of field use on these *Lygodium* species is good evidence that the field host-range of this insect may be limited to relatively few species in the genus. This species is a good candidate agent for biological control of *L. microphyllum*.

3.2.4. *Cataclysta camptozonale (Lepidoptera: Pyralidae)*

Cataclysta camptozonale Hampson is distributed from subtropical southeast Queensland and northern New South Wales. The climate in southeast Queensland and northern New South Wales is cool-subtropical. This area rarely experiences freezing temperatures, but winter minimums normally fall to 3–5 °C several nights each year. *C. camptozonale* from this part of its range may be better adapted to the cool winters of south Florida.

Cataclysta is not the correct genus for this species. *Cataclysta* is in the subfamily Nymphulinae, which includes mostly species feeding on aquatic plants, whereas *C. camptozonale* has been assigned to the Musotiminae, which includes mostly fern-feeding species. A new genus will be described by Alma Solis, USDA-ARS, Systematic Entomology Laboratory, Beltsville, MD, and Shen-Horn Yen, Department of Biology, Imperial College at Silwood Park, Ascot, England which will include *C. camptozonale* and *Cataclysta* sp. B.

Adults are cream colored with light brown bands and darker speckles along the margins of the hind wings and forewings that are approximately 8 mm in length. Eggs are yellow and laid singly or in small clusters, mostly on the new growth. Larvae are green with no visible setae. Early instar larvae skeletonize *L. microphyllum* leaves, with fourth instar larvae consuming entire leaves. *C. camptozonale* pupate above ground on the plant in a concealed location. The approximate developmental period from egg to adult at 27 °C is 21 days. Natural populations remain at very low, sometimes at undetectable levels throughout the year. A localized outbreak near Logan in southeast Queensland occurred in April 2000. High densities of the larvae were noted

Table 3
Preliminary host-range testing of three Pyralidae[a]

Plant species	*Neomusotima conspurcatalis* Warren	*Cataclysta camptozonale* Hampson	*Musotima* sp.
Lygodium microphyllum (Cav.) R. Br. (QLD)	++	++	+
Lygodium microphyllum (FL)	++	++	++
Lygodium japonicum (Thunb.) Sw.	+	+	++
Lygodium palmatum (Bernhardi) Swartz	+	++	+
Lygodium reticulatum Schkuhr	NT	++	NT
Lygodium oligostachyum (Willdenow) Desv.	NT	0	NT
Anemia adiantifolia (L.) Swartz	NT	0	0
Schizaea bifida Willdenow	NT	0	NT
Asplenium nidus L.	0	0	0
Asplenium scolopendrium L.	NT	0	NT
Cyathea cooperi (F.J. Muell.) Domin	0	0	0
Hypolepis muelleri N.A. Wakoef	NT	0	NT
Pteridium aquilinum (L.) Kuhn	0	0	0
Nephrolepis biserrata (Swartz) Schott	0	0	0
Polystichum acrostichoides (Michaux) Schott	NT	0	NT
Rumohra adiantifolormis (G. Forst.) Ching	0	0	0
Sticherus flabellatus (R. Br.) H. St. John	NT	0	NT
Lycopodiella cernuum (L.) Pic. Serm.	NT	0	NT
Ophioglossum petiolatum L.f.	NT	0	NT
Osmunda regalis L.	0	0	0
Ceratopteris thalictroides (L.) Brongniart	NT	0	NT
Phlebodium aureum (L.) J. Smith	0	0	0
Platycerium hillii T. Moore	NT	0	NT
Psilotum nudum (L.) Palisot de Beauvois	NT	0	NT
Acrostichum speciosum Willdenow	NT	0	NT
Adiantum capillus-veneris L.	0	0	0
Salvinia molesta D.S. Mitchell	0	0	0
Selaginella emmelliana F. Schmitz M. Moller	NT	0	NT
Thelypteris patens (Swartz) Small	NT	0	NT

[a] No-choice tests were conducted, (++) indicates full development, (+) marginal development, (0) no development, NT, not tested; QLD, Queensland; FL, Florida.

defoliating entire patches of *L. microphyllum*. Searches were conducted on two other fern species in the same habitat; *Blechnum indicum* Burm. f. and *Hypolepis mueller* N.A. Wakoef, and no *C. camptozonale* were observed.

Host-range testing indicated that *C. camptozonale* is a specialist at the genus level (Table 3). Females were induced to lay eggs on nine fern species outside the *Lygodium* genus. In all cases the first-instar larvae died shortly after hatching. In most instances it was observed that the larvae attempted to feed on the test plant but did not find it suitable. In the field, the host range appears to be limited to *L. microphyllum* and *L. reticulatum*. Both species are sympatric in north Queensland in the wet tropics. No other *Lygodium* species are present in the range of *C. camptozonale*. However, based on the potential cold hardiness and its narrow host range, this species appears to be a good candidate agent for biological control.

3.2.5. Cataclysta sp. B (Lepidoptera: Pyralidae)

Cataclysta sp. B was collected from two sites in the wet tropics of north Queensland from both *L. microphyllum* and *L. reticulatum*. Adults are very similar in appearance to *C. camptozonale* and distinguished by observation of the female genitalia (Yen and Solis, Imperial College, Ascot, UK and USDA-ARS, Washington, DC, unpublished data). Its biology is similar to that of *C. camptozonale*. It may also be a good candidate for biological control.

3.2.6. Musotima sp. (Lepidoptera: Pyralidae)

Musotima sp. has been collected in lowland tropical Malaysia, Singapore, and southern Thailand. Specimens from these areas had identical molecular profiles. The distribution of this species appears to be limited to the wet tropics of Southeast Asia. It has a similar biology and developmental time as *N. conspurcatalis*.

Musotima is not the correct genus for this species. A new genus will be described by Alma Solis (USDA-ARS) and Shen-Horn Yen (Imperial College at Silwood Park, Ascot, UK), which will include this species.

Preliminary host-range testing indicates that *Musotima* sp. is a genus-level specialist, as oviposition and complete development occurred only on *Lygodium* species (Table 3). Females were induced to lay eggs on four fern species outside the *Lygodium* genus, but none of the larvae survived past the first instar. In the field, *L. japonicum*, *L. flexuosum*, and *L. circinatum* were surveyed but no *Musotima* sp. were collected, nor were any signs of its distinctive damage visible. This may be evidence that the field host-range of this insect may be more narrowly specific below the genus level. This species is a good candidate agent for biological control of *L. microphyllum*, especially in tropical parts of south Florida along the east coast.

3.2.7. Neomusotima fuscolinealis (Lepidoptera: Pyralidae)

Neomusotima fuscolinealis Yoshiyasu was reared from *L. japonicum* in Japan, in Tokyo and the Mie Prefecture near Nagoya. Although this Japanese moth was previously known, its host plant was not (Yoshiyasu, 1985). It is a foliage-feeder and is believed to have a similar biology to *N. conspurcatalis*. The moth is probably a narrow specialist like other *Lygodium* feeding pyralids. *N. fuscolinealis* larvae were observed to feed upon and defoliate *L. japonicum* plants growing in the fern collection of the University of Tokyo Botanical Gardens, but no larvae or characteristic feeding damage (skeletonized leaves, silk webs with frass) were found on other fern species. Its molecular profile is distinct from those of the other Pyralidae in this survey. This temperate species may be useful if a biological control program is initiated for *L. japonicum*, if it is unable to use the temperate North American *L. palmatum* as a host.

3.2.8. Ambia sp. (Lepidoptera: Pyralidae)

An unknown stem-boring *Ambia* species was collected from *L. microphyllum* in Singapore and *L. flexuosum* in Thailand. Molecular profiles are considerably different for *Ambia* sp. from both locations and host plants, which most likely indicates the existence of two species. Although very similar in appearance, the Thai moth's white forewing is 8-mm long and patterned with black, which creates a strong resemblance to a crab-like spider, which it may mimic. The larvae can cause considerable damage to *L. microphyllum* as they tunnel in stems, eventually causing death of the shoot distal to the larval feeding. Although uncommon in the field, the sight of a long, dead shoot amongst undamaged healthy foliage was impressive and indicated the potential damage that could be caused by a large population of this pyralid. Larvae have a terminal, sclerotized plate, presumably to block the tunnel or the entry/exit hole in the stem. Stem damage has also been observed on *L. flexuosum* in Singapore. This species has been difficult to bring into culture for host-specificity testing because of its rarity. It is a good candidate agent for biological control because of the unique damage it causes to *L. microphyllum*.

3.2.9. Pyraustinae gen. nov. sp. nov. (Lepidoptera: Pyralidae)

An unknown foliage-feeding species of Pyraustinae was collected from *L. microphyllum* in New Caledonia. The adults are pale fuscous brown, with forewings approximately 7 mm in length. The larvae are green, skeletonizing leaves as early instars and consuming whole leaves in the later instars. At one site near Noumea in Province Sud, New Caledonia, this species was very abundant on two occasions, causing considerable damage to *L. microphyllum*. Collections were made during the day (mid-morning) and at night 1–2 h after sunset. Larvae were inconspicuous or hiding during the day and feeding on the vines on trees at night, suggesting that they appear to be nocturnal feeders. Many more larvae were collected in the night surveys. We did not collect any of the pyraustine larvae on the foliage of other ferns during day or night searches at this location, indicating that this species may have a narrow host range.

3.2.10. Callopistria spp. (Lepidoptera: Noctuidae)

Several species of *Callopistria* have been collected from *L. microphyllum* in Australia and Asia. Molecular diagnostics indicates three putative species: 'A' type from Australia (Queensland), China, India and Thailand; 'B' type from Australia (Northern Territory and Western Australia), and 'C' type from Thailand. All the known *Callopistria* spp. are fern-feeders, and most are thought to be generalist fern-feeders (Robinson, London Museum of Natural History, London, UK, personal communication), but little is known about the host range of the species collected from *L. microphyllum*. An American species, *Callopistria floridensis* Guenée occurs in the same habitat with *L. microphyllum* in Florida (Buckingham and Pemberton, USDA-ARS, Gainesville, Florida, unpublished data) and is known to be a generalist fern feeder and a pest of cultivated ferns in Florida (Leibee and Stamps, 1999). Limited testing of one *Callopistria* sp. in Australia suggested the possibility of greater specialization of *Lygodium*-feeding species encountered in our surveys. The presence of a congeneric *Callospistria* in Florida probably also means the presence of parasitoids of *Callopistria* which might influence the abundance of *Callopistria* spp. introduced for biological control of *L. microphyllum*.

3.2.10.1. Callopistria sp. A. *Callopistria* sp. A has been collected from *L. microphyllum* near Cairns, north Queensland, Australia; Hong Kong and Hainan, southern China; southern Tamil Nadu, India and several locations throughout Thailand. Molecular profiles for specimens from these locations are identical. The adult is dark grayish brown with a mosaic of black, light gray, and brown patches on its forewings. The forewings are 12 mm in length with distinctive light gray bands extending to the inner margins. The larvae are green as early instars, becoming pink in the last instar. Early instars skeletonize leaves and later consume whole leaves as they mature. Clusters of leaves with missing pinnae in a 'fish bone' pattern are characteristic of the larval feeding damage. This distinctive damage can be used to locate 'hot spots' of *Callopistria*.

3.2.10.2. Callopistria sp. B. *Callopistria* sp. B has been collected from the Kimberly Region near Kununurra in Western Australia and Litchfield National Park in Northern Territory. Molecular profiles are the same for both populations. The adults and immatures have a similar biology and appearance to *Callopistria* sp. A. The population from Western Australia was tested in two choice experiments. In the first experiment the *L. microphyllum* plus other non-target test species, *Anemia adiantifolia* (L.) Swartz, *Cyathea cooperi* (F.J. Muell.) Domin, *Thelypteris patens* (Swartz) Small, and *Adiantum capillus-veneris* L. were exposed to adult moths. In the second experiment the same non-target test species were caged without *L. microphyllum.* In the first experiment, large numbers of eggs were laid on *L. microphyllum*. Larvae in this test completely consumed the *L. microphyllum* plant, but no damage was observed on the other species. In the second experiment, eggs were laid on *C. cooperi*, but no development was observed. Further testing is needed to determine the host range of this species.

3.2.10.3. Callopistria sp. C. *Callopistria* sp. C has only been collected once from *L. microphyllum* in Thailand. The adults have a similar appearance to *Callopistria* sp. A. Further testing is needed to determine the host range of this species.

3.2.11. Spodoptera litura (Lepidoptera: Noctuidae)

Spodoptera litura (F.) has been collected from *L. microphyllum* in southeast Queensland. This species is highly polyphagous and is frequently a greenhouse pest at CSIRO research facilities in Indooroopilly, Queensland. It has also been reared from field collections on *Melaleuca quinquenervia* (Myrtaceae). This armyworm is a well-known pest of row crops, trees, and even aquatic plants in Asia (Robinson et al., 2001). This species is not being considered as a biological control agent because of its broad host range.

3.2.12. Archips machlopis (Lepidoptera: Tortricidae)

Archips machlopis Meyrick were collected from *L. microphyllum* at six sites in southern Thailand. This moth belongs to a species-group that is known to be polyphagous (Robinson et al., 2001). Therefore, it is not being considered for further testing.

3.2.13. Neostromboceros albicomus (Hymenoptera: Tenthridinidae)

Neostromboceros albicomus (Konow) was collected from *L. microphyllum* in Thailand and Vietnam and from *L. flexuosum* in Thailand. *Neostromboceros albicomus* from *L. flexuosum* and *L. microphyllum* may represent two separate species. Specimens apparently identical to the *L. flexuosum* sawfly (*N. albicomus*), but collected from *L. microphyllum*, were shown by DNA comparisons to be slightly different. Sequencing of the

D2 gene revealed a two base-pair difference. It is not known if this amount of genetic difference indicates that the two types are separate species. However, each type develops more readily on its original host species, *N. albicomus* A on *L. flexuosum*, and *N. albicomus* B on *L. microphyllum*. Smith et al. (2002) described specimens from both host plants as *N. albicomus*.

Adult *N. albicomus* are black and appear sluggish on the host plant. Females lay conspicuous yellow eggs, singly and in clusters, on the new growth. Larvae are bright yellow with an iridescent blue head and tip of abdomen. Local populations can reach fairly high densities, defoliating patches of *L. microphyllum* and *L. flexuosum*. Pupation occurs in the soil, which may provide protection from periodic wildfires. It appears from field surveys that 'B' form is specific to *L. microphyllum*. The apparently narrow host range of this insect and its unique biology as a sawfly make it a good candidate agent for biological control.

3.2.14. *Manobia sp. (Coleoptera: Chrysomelidae)*

The flea beetle, *Manobia* sp., was regularly collected from *L. flexuosum* in northern Thailand. Adults were observed feeding on the leaf margins of *L. flexuosum.* While rearing on *L. microphyllum* was not successful, the beetle reared well on *L. japonicum*. In quarantine, larvae appeared to be feeding on the roots and rhizome of *L. japonicum. L. japonicum* is reported to be weedy in central and north Florida and areas of the southern USA through to east Texas. If a biological control program is initiated for *L. japonicum*, this agent could be valuable if it cannot use the North American native *L. palmatum.*

3.2.15. *Endelus bakerianus (Coleoptera: Buprestidae)*

This leaf-mining beetle, *Endelus bakerianus* Obenberger, was collected in Singapore and Thailand from *L. microphyllum*. Females oviposit on the leaf margins of new growth and the larvae tunnel between leaf surfaces, completing development in one subpinna (leaflet). Pupation occurs in the leaf tissue. The length of the life cycle is not known. Although this species has only been collected from *L. microphyllum*, its field host range is not known. This species remains a candidate agent for biological control, but more information is needed on its potential for damage and its host range.

3.2.16. *Lophothetes sp. (Coleoptera: Apionidae)*

A defoliating species of *Lophothetes* was collected from *L. microphyllum* in Palau in the Pacific (V. Brancatini, CSIRO, Indooroopilly, Queensland, oral communication). Only adults were collected; no immatures were observed. Additional collections and testing are needed to determine its identity and host association with *L. microphyllum*.

3.2.17. *Acanthuchus trispinifer (Hemiptera: Membracidae)*

Acanthuchus trispinifer (Fairmaire) was collected in southeast Queensland and in the Northern Territory from *L. microphyllum*. Although some feeding damage was associated with adults, no reproduction was observed. Adults held in cages with whole plants lived for several weeks, but did not oviposit. *L. microphyllum* does not appear to be a primary host for *A. trispinifer*.

3.2.18. *Pseudococcus longispinus (Hemiptera: Pseudococcidae)*

Longtailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti), is highly polyphagous and a known pest of citrus and ornamentals (Clausen, 1978). This is the first record of this mealybug on *L. microphyllum*. This species is not being considered for further testing.

3.2.19. *Octothrips lygodii (Thysanoptera: Thripidae)*

Octothrips lygodii Mound was collected from *L. microphyllum*, *L. flexuosum*, and *L. japonicum* in China, Thailand, and Singapore (Mound, 2002). It is also described from Indonesia and Japan. This thrips is an extremely common herbivore in Southeast Asia, sometimes occurring in large numbers and being very damaging to *L. microphyllum*. The host range of *O. lygodii* collected in this survey is unknown.

3.3. *2000 Survey in India and Sri Lanka*

Preliminary surveys for herbivores were conducted in southern India and Sri Lanka in 2002. The west coast of Tamil Nadu from Chennai (Madras), over to wet, tropical Kerala and the highlands of the southern Ghat Mountains were explored. Two *Lygodium* species were found in this region, *L. flexuosum* and *L. microphyllum*, with the former being more common. A fern-feeding moth, *Callopistria* sp. A, was collected from two locations in Tamil Nadu along with the eriophyid, *F. perrepae*. In Sri Lanka, only *F. perrepae* was observed.

4. Discussion

Three fern species have been previously targeted in biological control programs: *Pteridium aquilinum* (L.) Kuhn (Kirk, 1977), *Salvinia molesta* Mitchell (Room et al., 1981), and *Azolla filiculoides* Lamarck (Hill, 1998). *P. aquilinum*, bracken fern, is a weed of agricultural and environmental areas (Kirk, 1977). It produces an array of arsensic-based compounds, which are both toxic and carcinogenic to cattle (Kirk, 1977). Exploration for agents of *P. aquilinum* was conducted over a two-year period in Papua New Guinea where the diversity of climates was expected to yield agents suitable for release in the UK and Australia. Kirk (1977) found

31 herbivores associated with bracken in the middle to higher elevations (450–3300 m) of its range in Papua New Guinea. However, worldwide more than 400 species of insects are recorded from *P. aquilinum* (Balik et al., 1978). None of the insect species collected from Papua New Guinea were released in the UK because of the concerns that biological control agents should not be released against a native plant species.

The second biological control program involved the aquatic fern *S. molesta*, giant salvinia. This program is now recognized as an example of one of the most successful examples of biological control efforts against a weed (Room et al., 1981). Three years of exploration were conducted in this plant's native range in south-eastern Brazil by Forno (1983) who documented 11 insect species feeding on *S. molesta*, with an additional 13 species believed to be herbivores but not confirmed, including *Cyrtobagous salviniae* Calder and Sands (Sands, 1983). The latter has successfully controlled the weed everywhere it has been released, including locations in Australia, Africa, and Asia (Forno, 1987; Julien et al., 2002; Sands et al., 1986).

The third fern species targeted for biological control is *A. filiculoides*, red water fern, which is native to South America and established in South Africa, where it is widespread, causing severe degradation of aquatic ecosystems (Hill, 1998). The weevil, *Stenopelmus rufinasus* Gyllenhal was released in South Africa to control red water fern, and initial results are promising (Hill, 1999). This weevil was originally collected from *Azolla caroliniana* Willd. in Florida. A short term, 9-day search in Argentina, the native range of *A. filculoides*, identified 3 herbivore species, including the allied weevil species *Stenopelmus brunneus* (Hustache).

The exploration efforts for the *P. aquilinum* and *S. molesta* program were similar, with 31 and 24 species of herbivores collected, respectively. The senior author queried the explorers of the previous three biological programs targeting ferns. All indicated that fern herbivores were rarely abundant in their native habitat, and that frequent searches were necessary to assess the species diversity.

The results of exploration for *L. microphyllum* agents follows the same trend that intensive studies are needed to assess their arthropod herbivore fauna. The number of herbivores collected in our study (21) is similar to the numbers collected in two of the three previous biological control programs targeting ferns. In field surveys, we found that because the insect herbivores of *L. microphyllum* occurred at such low density and were frequently below detection levels for long periods of time, frequent site-visits over several years were needed to collect the maximum number of herbivore species. This is quantified by the fact that in more than a quarter (26.3%) of the surveys, no herbivores were observed at all. Fig. 1 depicts the proportions of insects and mites collected from our surveys, which fall into the orders, Acarifomes, Lepidoptera, Coleoptera, Hymenoptera, Hemiptera, and Thysanoptera. In a review of the literature, Balik et al. (1978) found 420 arthropod species were recorded from ferns, of which the majority of insect herbivores were from the orders Coleoptera, Hymenoptera, Lepidoptera, and Hemiptera. Our survey tends to agree with Balik et al. (1978), but we found that Acarina and Lepidoptera formed the majority of herbivores collected, with Coleoptera and Hemiptera being by comparison under-represented. Lepidoptera species were represented in 43.2% of the collections. Of the Lepidoptera collected, 25% were species of *Callopistria*, a genus of specialist fern-feeders in the family Noctuidae. Of the remaining Lepidoptera, 75% were of fern-feeding Pyralidae, in the subfamily Musotiminae. This is similar to the other fern biological control programs, in which a total of five species of Pyralidae were collected. Of these five, only one, *Samea multiplicalis* Guenee (Pyraustinae), had sufficient host specificity to warrant release. In regard to *Lygodium*, all of the Musotiminae species appear to be genus-level specialists (Table 3). This suggests that the fern-specializing Musotiminae species may have a long evolutionary relationship with *Lygodium*. In contrast, Coleoptera were underrepresented in our survey, but dominant in the fern programs and well represented in biological control of weeds as a whole (Julien and Griffiths, 1998). In the *P. aquilinum* program, Coleoptera were dominant, and 18 species were collected in Papua New Guinea alone (Kirk, 1977). Weevils were the key biological control agents for the two aquatic ferns (Forno, 1987; Hill, 1999). Our survey also shows the lack of any Hemiptera. *Acanthuchus trispinifer* (Hemiptera) can be found occasionally feeding on *L. microphyllum*, but it probably does not use the plant for reproduction. Some families of Hemiptera are not common on ferns such as Aleyrodidae (Mound and Halsey, 1978) or Psyllidae, which do not utilize them at all (Dave Hollis, London Museum of Natural History, London, UK, personal communication). Only a few species of Hemiptera were discovered in the other fern biological control programs, so the lack of these species on *Lygodium* is not surprising. One possible explanation for the high numbers of Hemiptera recorded from ferns Balik et al. (1978) could be that they use the fern as a refuge rather than as a true host.

Why were so few insect species collected from *L. microphyllum*? Weather conditions often play a part in insect abundance, but our collections were conducted over 3 years across all seasons, which should have minimized the effect of weather on species diversity. We did see a trend that populations tended to be higher following rain, but overall species diversity did not change. Comparison of nutrient levels in *L. microphyllum* leaf tissues between Australia and Florida may provide some insight. Preliminary sampling suggests that the Australian *L. microphyllum* contains approximately 2/3 as much nitrogen, phosphorous, and potassium as found in the Florida plants (Goolsby, unpublished data). Low nutrient levels, particularly low nitrogen in the Australian *L. microphyllum*, may account for the low density of insects. Insect herbivores, particularly chewing insects, have longer development times, and thus greater exposure to both abiotic and biotic mortality when feeding on nitrogen-poor plants (Mattson, 1980). This suggests that the high-nitrogen *L. microphyllum* plants in Florida may support larger populations of lepidopteran biocontrol agents than their low-nitrogen Australian host. The addition of nitrogen to target weeds has been used to promote establishment and impact of lepidopteran natural enemies used in biological control of weeds (White, 1993). Balik et al. (1978) analyzed several fern species from the state of Veracruz, Mexico for the presence of tannins and cyanogenic glycosides, compounds known to be effective deterrents of herbivory in temperate climates. All the fern species contained tannins, but only 3% contained the cyanogenic glycosides, and the authors concluded that the compounds must not play a significant role as defensive compounds. *Lygodium* species probably have defense compounds, but we know of no studies that have investigated the presence or function of secondary plant defense compounds in *Lygodium* species. Another possible explanation for the low insect density could be the interaction between the eriophyid mite, *F. perrepae*, and the insect fauna. Mite-feeding appears to cause outbreaks of opportunistic fungi on the leaves, which causes premature senescence and leaf drop. Ovipositing insects may find the majority of the foliage on the mature stands of *L. microphyllum* to be unsuitable. Because the mite is nearly always associated with stands of the fern, an interaction of this sort could have a large-scale effect on insect herbivores. This effect could be relevant to multiple species releases. However, despite the low species richness as compared to higher plants, with intensive surveying we were able to detect a specialized fauna of some diversity, and importantly, natural enemies with biological control potential.

4.1. Future directions

The eriophyid mite, *F. perrepae*, should be given the highest priority as a biological control agent, based the damage it causes in the field and its potentially narrow host range. It is also the only herbivore that can be consistently collected in the field across all seasons and locations. Further, it is able to maintain populations in its native habitat a large suite of mite predators (Goolsby and Ozman, unpublished data). This would lead one to predict that this mite would be able to effectively deal with the predator complex in Florida, where *L. microphyllum* is invasive. The suite of leaf-feeding pyralid moths all appear to be narrowly specific, however several *Lygodium* species from the Caribbean will need to be tested. Additionally, the risk to the temperate *L. palmatum* by these tropical/subtropical moths will need to be assessed. In a few cases, we have observed outbreak populations of these species and confirmed that they can cause considerable damage to *L. microphyllum* stands (Goolsby, unpublished data). In the absence of co-adapted natural enemies, these moths may cause sustained damage. However, many factors affect establishment and success of Lepidoptera in biological control programs. Dray et al. (2001) discuss these factors in their unsuccessful attempts to establish a *Spodoptera pecticornis* Hampson (Lepidoptera: Noctuidae) against waterlettuce (*Pistia stratiotes* L.) in Florida. The *Callopistria* spp., also belonging to Noctuidae, may face similar challenges. The stem-boring pyralid, *Ambia* sp., should also be given high priority because of the nature of the damage it inflicts on the plant. Minor amounts of feeding could cause large portions of the vining fern to die. Further, the concealed habitat of this pyralid may protect it from attack by non-specialized parasitoids. The sawfly, *N. albicomus*, should also receive attention because of its narrow field host specificity and its ability to pupate in the soil, which may increase survival after wildfires in south Florida. Future surveys should target Southern Africa and India. Although the preliminary molecular data indicated that African populations of the fern were not a good match with Florida, additional herbivores with potential as biological control agents might be found there. The west coast of southern India (Kerala) has a moderately good climatic match with Florida and like Africa it is geographically distant from the search areas described in this paper.

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